TRISPECIFIC BINDING PROTEINS

I. SEQUENCE LISTING

[0001] This application contains a Sequence Listing, which has been submitted electronically in xml format and is hereby incorporated by reference in its entirety. Said xml copy, created on March 17, 2025, is named SeqList-350794-73000.xml and is 36,894 bytes in size.

II. FIELD OF THE INVENTION

[0002] The invention relates to trispecific binding proteins that bind to NKG2D, CD16, and cMET.

III. BACKGROUND

[0003] Cancer continues to be a significant health problem despite the substantial research efforts and scientific advances reported in the literature for treating this disease. Cancer immunotherapies are desirable because they are highly specific and can facilitate destruction of cancer cells using the patient's own immune system. Fusion proteins such as bi-specific T-cell engagers are cancer immunotherapies described in the literature that bind to tumor cells and T-cells to facilitate destruction of tumor cells. Antibodies that bind to certain tumor-associated antigens and to certain immune cells have been described in the literature. *See, e.g.,* WO 2016/134371 and WO 2015/095412.

[0004] Natural killer (NK) cells are a component of the innate immune system and make up approximately 15% of circulating lymphocytes. NK cells infiltrate virtually all tissues and were originally characterized by their ability to kill tumor cells effectively without the need for prior sensitization. Activated NK cells kill target cells by means similar to cytotoxic T cells — *i.e.,* via cytolytic granules that contain perforin and granzymes as well as via death receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN-γ and chemokines that promote the recruitment of other leukocytes to the target tissue.

[0005] NK cells respond to signals through a variety of activating and inhibitory receptors on their surface. For example, when NK cells encounter healthy self-cells, their activity is inhibited through activation of the killer-cell immunoglobulin-like receptors (KIRs). Alternatively, when NK cells encounter foreign cells or cancer cells, they are activated via their activating receptors *(e.g.,* NKG2D, NCRs, DNAM1). NK cells are also activated by the

constant region of some immunoglobulins through CD16 receptors on their surface. The overall sensitivity of NK cells to activation depends on the sum of stimulatory and inhibitory signals. NKG2D is a type-II transmembrane protein that is expressed by essentially all natural killer cells where NKG2D serves as an activating receptor. NKG2D is also found on T cells where it acts as a costimulatory receptor. The ability to modulate NK cell function via NKG2D is useful in various therapeutic contexts including malignancy.

IV. SUMMARY

[0006] Provided herein is a trispecific binding protein that binds to c-Met, NKG2D, and CD16. In some embodiments, each heavy chain of the trispecific binding protein comprises a terminal lysine when first produced, resulting in reduced terminal proline amidation following Protein A affinity chromatography, as compared to a trispecific binding protein but lacking the terminal lysine.

[0007] Also provided is a composition comprising a plurality of trispecific binding proteins, wherein the heavy chains of the trispecific binding proteins comprise a C-terminal prolinamide, a C-terminal PG dipeptide (proline-glycine), or a C-terminal PGK tripeptide. The composition, in embodiments, comprises not more than 1% of the heavy chains terminating with C-terminal prolinamide, at least 85% of the heavy chains terminating with C-terminal PG dipeptide, and at least 3% of the heavy chains terminating with C-terminal PGK tripeptide. In embodiments, the composition is a Protein A affinity chromatography purified composition of trispecific binding proteins.

[0008] In embodiments, the composition comprises a plurality of trispecific binding proteins, wherein the heavy chains of the trispecific binding proteins comprise a C-terminal proline, a C-terminal prolinamide, a C-terminal PG dipeptide (proline-glycine), or a C-terminal PGK tripeptide. The composition, in embodiments, comprises trispecific binding protein species in which not more than 0.1% of the heavy chains terminate with proline, not more than 1% of the heavy chains terminate with prolinamide, at least 85% of the heavy chains terminate with the PG dipeptide, and at least 3% of the heavy chains terminate with the C-terminal PGK tripeptide.

[0009] In embodiments, the amount of the C-terminal variants in the composition is determined using mass spectrometry.

[0010] In embodiments, the composition comprises a trispecific binding protein in which the first and second heavy chains that form an Fc region have different C-terminal amino acids or a

trispecific binding protein in which the first and second heavy chains that form an Fc region have the same C-terminal amino acids, including compositions comprising a plurality of trispecific binding proteins in which the first and second heavy chains that form an Fc region in some of the trispecific binding proteins have different C-terminal amino acids and the first and second heavy chains that form an Fc region in some of the trispecific binding proteins have the same C-terminal amino acids.

[0011] Various aspects and embodiments are described in further detail below.

V. BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1 represents a trispecific antibody that contains a cMet tumor-targeting scFv, a NKG2D-targeting Fab fragment, and a heterodimerized antibody Fc region that binds CD16. Heterodimerization mutations on the antibody CH3 domain include K360E and K409W on one CH3 domain and Q347R, D399V, and F405T on the opposite CH3 domain (shown as a triangular lock-and-key shape in the CH3 domains); and Y349C on one constant domain and S354C on the opposition constant domain (shown as a bold bar between the CH3 domains) according to Kabat numbering.

VI. DETAILED DESCRIPTION

[0013] The invention provides trispecific binding proteins that bind c-Met, NKG2D, and CD16. The invention also provides compositions comprising these trispecific binding proteins, and therapeutic methods using these trispecific binding proteins and compositions for treating cancer.

[0014] Provided herein is an improvement on a single-chain variable fragment (scFv) that is linked to an antibody constant domain via a hinge sequence. The improvement is directed to the inclusion of terminal lysines at the C terminus of the heavy chains, which reduces the occurrence of a problematic production variant terminating in an amindated proline (prolinamide); Jiang et al., Journal of Pharmaceutical Sciences 105 (2016) 2066-2072.

[0015] As used in this disclosure, the singular forms “a”, “an” and “the” include plural referents unless the context clearly dictates otherwise. The terms “a” (or “an”), as well as the terms “one or more,” and “at least one” can be used interchangeably herein unless the context clearly dictates otherwise.

[0016] As used in this disclosure and unless otherwise specified, the terms “about” and “approximately” generally refer to a range of numbers that one of skill in the art would consider equivalent to the recited value (i.e., having the same function or result). In many

instances, the terms “about” and “approximately” may include numbers that are rounded to the nearest significant figure. In specific embodiments, the terms “about” and “approximately” shall be construed so as to allow normal variation as judged by a person of ordinary skill in the art, such as, for example, a variation within 20% or 10% or 5%. In specific embodiments, the terms “about” and “approximately” encompass the exact value recited. Unless the context clearly dictates otherwise, all numerical values provided herein are modified by the term about.

[0017] As used herein, "c-MET" (also known as HGFR; AUTS9; RCCP2; and c-Met; DFNB97) refers to the protein of Uniprot Accession No. P08581 and related isoforms.

A. Trispecific Binding Proteins

[0018] Provided herein are trispecific binding proteins that bind c-Met, NKG2D, and CD16. Binding of the trispecific binding proteins to NKG2D and CD16 on a natural killer cell enhances the activity of the natural killer cell toward destruction of tumor cells expressing c-MET. Binding of the trispecific binding proteins to tumor-associated antigen-expressing cells brings the cancer cells into proximity with the natural killer cell, which facilitates direct and indirect destruction of the cancer cells by the natural killer cell.

[0019] The first component of the trispecific binding proteins is a Fab that binds to NKG2D receptor-expressing cells, which can include but are not limited to NK cells, γδ T cells and $CD8^+$ αβ T cells. Upon NKG2D binding, the trispecific binding proteins may block natural ligands, such as ULBP6 and MICA, from binding to NKG2D and activating NK cells.

[0020] The second component of the trispecific binding proteins is an scFv that binds c-MET. c-MET-expressing cells may be found in various cancers, including non-small cell lung cancer, renal cell carcinoma, and head and neck squamous cell carcinoma.

[0021] The third component of the trispecific binding proteins is an Fc region that binds to cells expressing CD16, an Fc receptor on the surface of leukocytes including natural killer cells, macrophages, neutrophils, eosinophils, mast cells, and follicular dendritic cells.

[0022] The trispecific proteins herein are heterodimeric, comprising a first heavy chain, a light chain, and a second heavy chain (FIG. 1). The first heavy chain comprises a heavy chain variable domain, a CH1 domain, and a first Fc region (hinge-CH2-CH3). The light chain comprises a light chain variable domain and a light chain constant region. The first heavy chain pairs with the light chain to form a Fab that binds NKG2D. The second heavy chain comprises a single-chain variable fragment (scFv) that binds c-MET and a second Fc region. The first and second heavy chains pair to form an Fc region capable of binding CD16.

[0023] The first and second heavy chains of the trispecific binding proteins herein are engineered to include a terminal lysine. The present application is directed to the surprising finding that inclusion of the terminal lysine reduces the occurrence of a problematic production variant terminating in an amidated proline (prolinamide).

[0024] The Fc region of the trispecific proteins herein have further been engineered to be of the z, non-a allotype. Two amino acid substitutions (D356E, L358M) present in the heavy chains of Ab1 provide the z, non-a allotype.

[0025] In some embodiments, the trispecific binding protein comprises a first heavy chain, a light chain and a second heavy chain. In some embodiments, the first heavy chain comprises, from N-terminus to C-terminus: a heavy chain variable domain of a Fab fragment that binds NKG2D, a CH1 domain, a first CH2 domain and a first CH3 domain. In some embodiments, the light chain comprises from N-terminus to C-terminus: a light chain variable domain of the Fab fragment that binds NKG2D and a CL domain. In some embodiments, the second heavy chain comprises, from N-terminus to C-terminus: an scFv that binds cMET, a second CH2 domain and a second CH3 domain. In some embodiments, the first heavy chain and light chain form a Fab that binds NKG2D, wherein the second heavy chain comprises an scFv that binds cMET, and wherein the first and second heavy chains comprise a first and a second CH2 and CH3 domain that form an Fc region that binds CD16.

[0026] In some embodiments, the first heavy chain comprises the amino acid sequence shown as SEQ ID NO: 5 (variable region is **bold** and has the amino acid sequence shown as SEQ ID NO: 1, CDRs are underlined and have the amino acid sequences shown as SEQ ID NOs: 8, 9, and 10, respectively, in order of appearance; constant region is *italicized,* two individual z-non-a residues are underlined):

EVQLVESGGGLVKPGGSLRLSCAAS<u>GFTFSSY</u>SMNWVRQAPGKGLEWVSSI<u>SSSS SY</u>IYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR<u>GAPIGAAAGWF DP</u>WGQGTLVTVSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV CTLPPSR<u>E</u>E<u>M</u>TENQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSWL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO: 5).

[0027] In some embodiments, the light chain comprises the amino acid sequence shown as SEQ ID NO: 6 (variable region is **bold** and has the amino acid sequence shown as SEQ ID NO:

2, CDRs are underlined and have amino acid sequences shown as SEQ ID NOs: 11, 12, and 13, respectively, in order of appearance; constant region is *italicized*):

DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGVSFPRTFGGGTKVEIK*RTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO: 6).

[0028] In some embodiments, the second heavy chain comprises the amino acid sequence shown as SEQ ID NO: 7 (light chain variable region is **bold** and has the amino acid sequence shown as SEQ ID NO:4, light chain CDRs are underlined and have amino acid sequences shown as SEQ ID Nos: 17, 18, and 19, respectively in order of appearance; heavy chain variable region is ***bold italicized*** and has the amino acid sequence shown as SEQ ID NO: 3, heavy chain CDRs are underlined and have amino acid sequences shown as SEQ ID Nos: 14, 15, and 16, respectively, in order of appearance; (G4S)4 linker has no bolding, italicizing, or underlining and has the amino acid sequence shown as SEQ ID NO: 22; constant region is *italicized,* two individual z-non-a residues are underlined):

DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQQKPGQPPKLLIYRAS TRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSKEDPLTFGCGTKVEIKG GGGSGGGGSGGGGSGGGGS***QVQLVQSGAEVKKPGASVKVSCKASGYIFTAYTMHWV RQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAV YYCARSEITTEFDYWGQGTLVTVSS***AS*DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPRVYTLPPCREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK* (SEQ ID NO: 7).

[0029] The single-chain variable fragment (scFv) described above is linked to the antibody constant domain via a linker sequence comprising amino acids Ala-Ser. The linker sequence can provide flexibility of binding to the target antigen, and balance between flexibility and optimal geometry.

[0030] The single-chain variable fragment (scFv) described above includes a heavy chain variable domain and a light chain variable domain. In some embodiments, the heavy chain variable domain forms a disulfide bridge with the light chain variable domain to enhance stability of the scFv. For example, a disulfide bridge can be formed between the C44 residue of the heavy chain variable domain and the C100 residue of the light chain variable domain, the

amino acid positions numbered under Kabat. In some embodiments, the heavy chain variable domain is linked to the light chain variable domain via a flexible linker. Any suitable linker can be used, for example, the (G4S)4 linker (SEQ ID NO: 22). In some embodiments of the scFv, the heavy chain variable domain is positioned at the N-terminus of the light chain variable domain. In some embodiments of the scFv, the heavy chain variable domain is positioned at the C terminus of the light chain variable domain.

[0031] In some embodiments, a heterodimeric Fc region is formed by heterodimerization between a first heavy chain constant region comprising a first CH2CH3 comprising Y349C, K360E, and K409W substitutions and a second heavy chain constant region comprising a second CH2CH3 comprising S354C, Q347R, D399V, and F405T substitutions.

[0032] Within the Fc region, CD16 binding is mediated by the hinge region and the CH2 domain. For example, within human IgGl, the interaction with CD16 is primarily focused on amino acid residues Asp 265 - Glu 269, Asn 297 - Thr 299, Ala 327 - Ile 332, Leu 234 - Ser 239, and carbohydrate residue N-acetyl-D-glucosamine in the CH2 domain (see, Sondermann *et al.,* Nature, 406 (6793):267-273).

[0033] The trispecific binding proteins described above can be made using recombinant DNA technology well known to a skilled person in the art. For example, a first nucleic acid sequence encoding the first immunoglobulin heavy chain can be cloned into a first expression vector; a second nucleic acid sequence encoding the second immunoglobulin heavy chain can be cloned into a second expression vector; a third nucleic acid sequence encoding the immunoglobulin light chain can be cloned into a third expression vector; and the first, second, and third expression vectors can be stably transfected together into host cells to produce the multimeric proteins.

B. Compositions Comprising Trispecific Binding Proteins

[0034] In some aspects, provided herein are compositions comprising a mixture of trispecific binding proteins, wherein when first produced, the trispecific binding protein comprises a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 5; a light chain comprising the amino acid sequence shown as SEQ ID NO: 6; and a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 7; wherein the first heavy chain and the light chain form a Fab that binds NKG2D, wherein the second heavy chain comprises an scFv that binds cMET, and wherein the first and second heavy chains form an Fc region that binds CD16.

[0035] Compositions comprising antibodies are heterogeneous, comprising various variant antibody species that arise during production and purification. For example, antibody heavy chains terminating in lysine ("LSPGK variants") are known to give rise to variant species through clipping or cleavage of the terminal lysine, resulting in heavy chains terminating in glycine ("LSPG variants"). In some instances, the terminal glycine is clipped, resulting in a terminal proline, which is frequently amidated in the process, converting the proline to prolinamide. Amidated proline variants ("LSP[amide] variants") have been shown to have an unpredictable effect on antibody stability, in contrast to the well understood LSPGK and LSPG variants.

[0036] The heavy chains of the trispecific binding proteins described herein comprise a C-terminal lysine when first produced. The present application is directed in part to the surprising finding that trispecific binding proteins produced with a terminal lysine results in a lower percentage of variant species terminating in an amidated proline following Protein A affinity chromatography purification as compared to a version engineered to lack a heavy chain terminal lysine.

[0037] In some embodiments, provided herein are compositions comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins. In some embodiments, each trispecific protein comprises a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26; a light chain comprising the amino acid sequence shown as SEQ ID NO: 6; and a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30; wherein the first heavy chain and the light chain form a Fab that binds NKG2D, the second heavy chain comprises an scFv that binds cMET, and the first and second heavy chains form an Fc region; and wherein the first and/or second heavy chain of each trispecific protein further comprises one of an additional C-terminal prolinamide, an additional C-terminal proline-glycine dipeptide, or an additional C-terminal proline-glycine-lysine tripeptide. In some embodiments, not more than 1% of the heavy chains in the composition comprise a C-terminal prolinamide, at least 85% of the heavy chains in the composition comprise a C-terminal proline-glycine dipeptide, and at least 3% of the heavy chains comprise a C-terminal proline-glycine-lysine tripeptide.

[0038] In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26, further comprising an additional C-terminal proline-glycine (PG) dipeptide. In some embodiments, the first heavy

chain comprises the amino acid sequence shown as SEQ ID NO: 29 (SEQ ID NO: 26 with C-terminal PG dipeptide). In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30, further comprising an additional C-terminal PG dipeptide. In some embodiments, the second heavy chain comprises the amino acid sequence shown as SEQ ID NO: 33 (SEQ ID NO: 30 with C-terminal PG dipeptide). In some embodiments, at least 85% of the heavy chains in the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal PG dipeptide.

[0039] In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26, further comprising an additional C-terminal proline-glycine-lysine (PGK) tripeptide. In some embodiments, the first heavy chain comprises the amino acid sequence shown as SEQ ID NO: 5 (SEQ ID NO: 26 with C-terminal PGK tripeptide). In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30, further comprising an additional C-terminal PGK tripeptide. In some embodiments, the second heavy chain comprises the amino acid sequence shown as SEQ ID NO: 7 (SEQ ID NO: 30 with C-terminal PGK tripeptide). In some embodiments, at least 3% of the heavy chains in the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal PGK tripeptide. In embodiments, at least 4%, 5%, 6% or 8% of the heavy chains in the composition comprising a Protein-A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal PGK tripeptide. In embodiments, not more than 11% of the heavy chains in the composition comprising a Protein-A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal PGK tripeptide.

[0040] In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26, further comprising an additional C-terminal prolinamide. In some embodiments, the first heavy chain comprises the amino acid sequence shown as SEQ ID NO: 28 (SEQ ID NO: 26 with C-terminal prolinamide). In some embodiments, the composition comprising a Protein A affinity chromatography

purified mixture of trispecific binding proteins comprises a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30, further comprising an additional C-terminal prolinamide. In some embodiments, the second heavy chain comprises the amino acid sequence of SEQ ID NO: 32 (SEQ ID NO: 30 with C-terminal prolinamide). In some embodiments, not more than 1% of the heavy chains in the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal prolinamide. In some embodiments, not more than 0.8%, 0.5%, 0.3%, 0.2% or 0.1% of the heavy chains in the composition comprising a Protein-A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal prolinamide.

[0041] In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26, further comprising an additional C-terminal proline. In some embodiments, the first heavy chain comprises the amino acid sequence shown as SEQ ID NO: 27 (SEQ ID NO: 26 with C-terminal proline). In some embodiments, the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins comprises a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30, further comprising an additional C-terminal proline. In some embodiments, the second heavy chain comprises the amino acid sequence shown as SEQ ID NO: 31 (SEQ ID NO: 30 with C-terminal prolinamide). In some embodiments, not more than 0.1% of the heavy chains in the composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal proline. In some embodiments, not more than 0.08%, 0.05%, 0.03%, 0.02% or 0.01% of the heavy chains in the composition in the composition comprising a Protein-A affinity chromatography purified mixture of trispecific binding proteins terminate with the C-terminal proline.

[0042] In some embodiments, at least 3% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the PGK tripeptide following protein A purification. In some embodiments, at least 4% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the PGK tripeptide following Protein A purification. In some embodiments, at least 5% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the PGK tripeptide following protein A purification. In some embodiments, at least 6% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the

PGK tripeptide following Protein A purification. In some embodiments, at least 7% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the PGK tripeptide following protein A purification. In some embodiments, at least 8% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the PGK tripeptide following Protein A purification. In some embodiments, at least 9% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in the PGK tripeptide following protein A purification.

[0043] In some embodiments, not more than 1% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in prolinamide following protein A purification. In some embodiments, not more than 0.8% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in prolinamide following protein A purification. In some embodiments, not more than 0.6% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in prolinamide following protein A purification. In some embodiments, not more than 0.4% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in prolinamide following protein A purification. In some embodiments, not more than 0.2% of the first and/or the second heavy chain of the trispecific binding protein in the composition terminates in prolinamide following protein A purification. In some embodiments, the terminal proline corresponds to P445 of IgG1, according to Kabat numbering.

[0044] The percentage of the first and/or the second heavy chain of the trispecific binding protein terminating in an amidated proline can be determined by methods known in the art. In some embodiments, the percentage of the first and/or the second heavy chain of the trispecific binding protein in the composition terminating in an amidated proline is determined using mass spectrometry.

C. Therapeutic Applications

[0045] The invention provides methods for treating cancer using a trispecific binding protein described herein and/or a pharmaceutical composition described herein. The methods may be used to treat a variety of cancers expressing c-MET.

[0046] Exemplary cancers to be treated by the c-MET-targeting trispecific binding proteins may be renal cancer, thyroid cancer, melanoma, lung cancer, melanoma, liver cancer, pancreatic cancer, colorectal cancer, and head and neck cancer.

VII. EXAMPLES

[0047] The invention now being generally described will be more readily understood by reference to the following example, which is included merely for purposes of illustration of certain aspects and embodiments of the present invention and is not intended to limit the invention.

Example 1:

[0048] Antibody manufacturing via cell culture produces heterogeneous variants which can accumulate during production, purification, formulation, and storage. These variants can have reduced stability, which can pose a safety and potency concern. One such production variant terminates with prolinamide on heavy chains (Jiang et al., Journal of Pharmaceutical Sciences 105 (2016) 2066-2072).

[0049] Ab1 is a z, non-a allotype trispecific binding protein with a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 5, a light chain comprising the amino acid sequence shown as SEQ ID NO: 6, and a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 7. Ab2 is nearly identical, differing at 3 residues in the heavy chains. The heavy chains of Ab1 (SEQ ID NOs: 5 and 7) includes a C-terminal lysine absent in the heavy chains of Ab2 (SEQ ID NOs: 24 and 25). In addition, two amino acid substitutions (D356E, L358M) present in the heavy chains of Ab1, but not in those of Ab2, provide the z, non-a allotype. Both Ab1 and Ab2 have a light chain comprising the amino acid sequence shown as SEQ ID NO: 6.

[0050] Ab1 and Ab2 were purified by Protein A affinity chromatography. Following tryptic digests, the two antibodies were analyzed by reverse-phase HPLC with online mass spectrometry. Peptides were identified by accurate mass determination to within ±7 ppm of the theoretical values from the expected amino acid sequence. Peak areas of ions representing C-terminal heavy-chain peptide variants (LSPGK, LSPG, LSP, LSP[amide]) were used to derive relative level of each variant species.

[0051] As shown in Table 1, the majority of both Ab1 and Ab2 exist as the LSPG variant. About 98.5% of Ab2 exists as the LSPG variant, however the remainder of Ab2 in the composition undergoes a further cleavage of the terminal glycine and amidation of the remaining terminal proline, resulting in about 1.50% of the composition existing as the LSP[amide] variant. In contrast, only a small proportion of Ab1 exists as the LSP[amide] variant, with the second most abundant variant being the LSPGK.

Table 1

Sample	LSPGK (%)	LSPG (%)	LSP (%)	LSP[amide] (%)
Ab2	0.01	98.52	0.00	1.47
Ab1 (clone 1)	5.37	94.52	0.00	0.11
Ab1 (clone 2)	6.83	93.04	0.01	0.12
Ab1 (clone 3)	9.09	89.94	0.01	0.96
Ab1 (clone 4)	10.02	89.44	0.01	0.53
Ab1 (clone 5)	3.58	96.26	0.02	0.14

INCORPORATION BY REFERENCE

[0052] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0053] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. The scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE TABLE

SEQ ID NO:	Sequence	Description
1	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS S	NKG2D VH
2	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQGVSFPRTFGGGTKVEIK	NKG2D VL
3	QVQLVQSGAEVKKPGASVKVSCKASGYIFTAYTMHWVRQ APGQ**C**LEWMGWIKPNNGLANYAQKFQGRVTMTRDTSIST AYMELSRLRSDDTAVYYCARSEITTEFDYWGQGTLVTVSS	cMET VH
4	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFG**C**GTKVEIK	cMET VL
5	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS S*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS*	NKG2D VH-CH1-CH2-CH3

	GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTENQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	
6	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQGVSFPRTFGGGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	NKG2D VL-CL
7	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSSASDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPRVYTLPPCREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	cMET scFv-CH2-CH3
8	GFTFSSY	NKG2D CDR-H1
9	SSSSSY	NKG2D CDR-H2
10	GAPIGAAAGWFDP	NKG2D CDR-H3
11	RASQGISSWLA	NKG2D CDR-L1
12	AASSLQS	NKG2D CDR-L2
13	QQGVSFPRT	NKG2D CDR-L3
14	GYIFTAY	cMET CDR-H1
15	KPNNGL	cMET CDR-H2
16	SEITTEFDY	cMET CDR-H3
17	KSSESVDSYANSFLH	cMET CDR-L1
18	RASTRES	cMET CDR-L2
19	QQSKEDPLT	cMET CDR-L3
20	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVCTLPPSREEMTENQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK	NKG2D Fc
21	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPRVYTLPPCREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK	cMET Fc

22	GGGGSGGGGSGGGGSGGGGS	(G4S)4 linker
23	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSS	c-MET full scFv
24	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTE NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSWLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG	Ab2 anti- NKG2D VH- CH1-CH2-CH3
25	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSSASDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPRVYTLPPCRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	Ab2 anti-c-Met scFv-CH2-CH3
26	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS S*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS* *GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK* *PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK* *DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK* *PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE* *KTISKAKGQPREPQVCTLPPSREEMTENQVSLTCLVKGFYPSDI* *AVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQ* *GNVFSCSVMHEALHNHYTQKSLSLS*	NKG2D VH- CH1-CH2-CH3 LS
27	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS S*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS* *GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK* *PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK* *DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK*	NKG2D VH- CH1-CH2-CH3 LSP Variant

	PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTENQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSP	
28	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS S*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTENQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSP* Note: terminal proline is prolinamide	NKG2D VH-CH1-CH2-CH3 LSP Prolinamide Variant
29	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQA PGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLVTVS S*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSREEMTENQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG*	NKG2D VH-CH1-CH2-CH3 LSPG Variant
30	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSSASDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPRVYTLPPCREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLS	cMET scFv-CH2-CH3 LS
31	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSSASDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPRVYTLPPCREEMTKNQVSLTCLVKGFYP	cMET scFv-CH2-CH3 LSP Variant

	SDIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSP	
32	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSSASDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPRVYTLPPCREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSP Note: terminal proline is prolinamide	cMET scFv-CH2-CH3 LSP Prolinamide Variant
33	DIVMTQSPDSLAVSLGERATINCKSSESVDSYANSFLHWYQ QKPGQPPKLLIYRASTRESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYYCQQSKEDPLTFGCGTKVEIKGGGGSGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYIFTAY TMHWVRQAPGQCLEWMGWIKPNNGLANYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARSEITTEFDYWGQG TLVTVSSASDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPRVYTLPPCREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	cMET scFv-CH2-CH3 LSPG Variant

WHAT IS CLAIMED IS:

1. A trispecific binding protein comprising:
    a. a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 5;
    b. a light chain comprising the amino acid sequence shown as SEQ ID NO: 6; and
    c. a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 7;

wherein the first heavy chain and the light chain form a Fab that binds NKG2D, wherein the second heavy chain comprises an scFv that binds cMET, and wherein the first and second heavy chains form an Fc region.

2. A composition comprising a plurality of trispecific binding proteins,
    a. wherein each trispecific binding protein comprises
        i. a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26;
        ii. a light chain comprising the amino acid sequence shown as SEQ ID NO: 6; and
        iii. a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30;
    b. wherein the heavy chains further comprise
        i. an additional C-terminal prolinamide (SEQ ID NO: 28 or SEQ ID NO: 32),
        ii. an additional C-terminal PG dipeptide (proline-glycine) (SEQ ID NO: 29 or SEQ ID NO: 33), or
        iii. an additional C-terminal PGK tripeptide (proline-glycine-lysine) (SEQ ID NO: 5 or SEQ ID NO: 7);
    c. wherein
        i. not more than 1% of the heavy chains terminate with prolinamide (SEQ ID NO: 28 or SEQ ID NO: 32);
        ii. at least 85% of the heavy chains terminate with the PG dipeptide (SEQ ID NO: 29 or SEQ ID NO: 33),

iii. at least 3% of the heavy chains terminate with the C-terminal PGK tripeptide (SEQ ID NO: 5 or SEQ ID NO: 7).

3. The composition of claim 2,
    a. wherein the heavy chains comprise
        i. an additional C-terminal proline (SEQ ID NO: 27 or SEQ ID NO: 31);
        ii. an additional C-terminal prolinamide (SEQ ID NO: 28 or SEQ ID NO: 32),
        iii. an additional C-terminal PG dipeptide (proline-glycine) (SEQ ID NO: 29 or SEQ ID NO: 33), or
        iv. an additional C-terminal PGK tripeptide (proline-glycine-lysine) (SEQ ID NO: 5 or SEQ ID NO: 7);
    b. wherein
        i. not more than 0.1% of the heavy chains terminate with proline (SEQ ID NO: 27 or SEQ ID NO: 31),
        ii. not more than 1% of the heavy chains terminate with prolinamide (SEQ ID NO: 28 or SEQ ID NO: 32),
        iii. at least 85% of the heavy chains terminate with the PG dipeptide (SEQ ID NO: 29 or SEQ ID NO: 33), and
        iv. at least 3% of the heavy chains terminate with the C-terminal PGK tripeptide (SEQ ID NO: 5 or SEQ ID NO: 7).

4. A composition comprising a Protein A affinity chromatography purified mixture of trispecific binding proteins,
    a. wherein each trispecific binding protein comprises
        i. a first heavy chain comprising the amino acid sequence shown as SEQ ID NO: 26;
        ii. a light chain comprising the amino acid sequence shown as SEQ ID NO: 6; and
        iii. a second heavy chain comprising the amino acid sequence shown as SEQ ID NO: 30;
    b. wherein the heavy chains comprise
        i. an additional C-terminal prolinamide (SEQ ID NO: 28 or SEQ ID NO: 32), or

ii. an additional C-terminal PG dipeptide (proline-glycine) (SEQ ID NO: 29 or SEQ ID NO: 33);

c. wherein
   i. the first heavy chain and the light chain form a Fab that binds NKG2D,
   ii. the second heavy chain comprises an scFv that binds cMET, and
   iii. the first and second heavy chains form an Fc region;

d. wherein
   i. not more than 1% of the heavy chains terminate in prolinamide (SEQ ID NO: 28 or SEQ ID NO: 32); and
   ii. at least 85% of the heavy chains terminate with the PG dipeptide (SEQ ID NO: 29 or SEQ ID NO: 33).

5. The composition of claims 2-4, wherein whether not more than 1% of the first and/or the second heavy chains in the composition terminate in prolinamide is determined using mass spectrometry.

6. The composition of claims 2-4, wherein whether at least 3% of the first and/or the second heavy chains in the composition terminate with the PGK tripeptide is determined using mass spectrometry.

7. The composition of claims 2-4, wherein the mixture comprises a trispecific binding protein in which the first and second heavy chains that form an Fc region have different C-terminal amino acids.

8. The composition of claims 2-4, wherein the mixture comprises a trispecific binding protein in which the first and second heavy chains that form an Fc region have the same C-terminal amino acids.

9. The composition of claims 2-4, wherein the mixture comprises a trispecific binding protein in which the first and second heavy chains that form an Fc region have different C-terminal amino acids and a trispecific binding protein in which the first and second heavy chains that form an Fc region have the same C-terminal amino acids.